Spatial and temporal variation of domoic acid in molluscs and of *Pseudo-nitzschia* spp. blooms in the St. Lawrence from 1998 to 2000

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ABSTRACT

Couture, J.Y., M. Levasseur, E. Bonneau, C. Desjardins, G. Sauvé, S.S. Bates, C. Léger, R. Gagnon and S. Michaud. 2001. Spatial and temporal variation of domoic acid in molluscs and of *Pseudo-nitzschia* spp. blooms in the St. Lawrence from 1998 to 2000. Can. Tech. Rep. Fish. Aquat. Sci. 2375: vii + 24 p.

The presence of domoic acid (the toxin responsible for Amnesic Shellfish Poisoning—ASP) in molluscs has been monitored in the estuary and the northern Gulf of St. Lawrence since 1997 by the Canadian Food Inspection Agency (CFIA). The results indicate a spatial and temporal evolution of domoic acid contamination between 1998 and 2000. Trace amounts of this toxin were first detected in the gonads of scallops from fishing areas offshore of the Îles-de-la-Madeleine in the summer of 1998. In 1999, the concentration of domoic acid in the digestive glands of scallops from the same area reached 585 $\mu g\ g^{-1}$ whereas the adductor muscles were not contaminated. At the same time, concentrations of domoic acid close to 25 $\mu g\ g^{-1}$ digestive gland were measured in scallops from the Havre-aux-Maisons lagoon while trace amounts were measured for the first time in soft-shell clams collected on the Basse-Côte-Nord of the Gulf of St. Lawrence. In 2000, the digestive glands of scallops from the Îles-de-la-Madeleine remained toxic and trace amounts of domoic acid were measured in molluscs all along the Côte-Nord, from Tadoussac to Havre-Saint-Pierre. In addition to the CFIA data, the Harmful Algae Monitoring Program of the Maurice Lamontagne Institute (Fisheries and Oceans Canada) revealed the presence of two potentially domoic-acid-producing diatoms in the St Lawrence: *Pseudo-nitzschia seriata* and *Pseudo-nitzschia delicatissima*. Analysis of data showed a link between domoic acid in some molluscs from the Îles-de-la-Madeleine and the Côte-Nord and the presence of *Pseudo-nitzschia seriata*. Dense blooms of *P. delicatissima* (with no *P. seriata*) did not cause toxicity. Laboratory analyses performed on a *P. seriata* strain isolated from the St. Lawrence estuary during a toxic event showed the ability of *P. seriata* to produce domoic acid whereas all attempts made with *P. delicatissima* from other regions of eastern Canada have so far been negative. These new results show that *P. seriata* blooms in the St. Lawrence and the resulting mollusc toxicity due to domoic acid represent a potential risk that needs to be considered in the future.

RÉSUMÉ

Couture J.Y., M. Levasseur, E. Bonneau, C. Desjardins, G. Sauvé, S.S. Bates, C. Léger, R. Gagnon et S. Michaud. 2001. Variations spatiales et temporelles des concentrations d'acide domoïque dans les mollusques et des abondances de *Pseudo-nitzschia* spp. dans le Saint-Laurent de 1998 à 2000. Rapp. tech. can. sci. halieut. aquat. 2375 : vii + 24 p.

La présence dans les mollusques de l'acide domoïque (toxine responsable de l'intoxication amnestique par les mollusques—IAM) est suivie dans l'estuaire et le nord du golfe du Saint-Laurent depuis 1997 par l'Agence Canadienne d'Inspection des Aliments (ACIA). Les résultats indiquent une évolution spatiale et temporelle de la contamination par l'acide domoïque au cours des années 1998 à 2000. Des traces de cette toxine ont d'abord été détectées dans des gonades de pétoncles pêchés au large des Îles-de-la-Madeleine à l'été 1998. En 1999, les concentrations d'acide domoïque dans les glandes digestives de pétoncles du même secteur atteignaient 585 $\mu g\, g^{-1}$, bien que les muscles adducteurs n'étaient pas contaminés. Pendant la même période, des teneurs de près de 25 $\mu g\, g^{-1}$ de glande digestive étaient mesurées dans des pétoncles récoltés dans la lagune de Havre-aux-Maisons, alors que des concentrations traces étaient mesurées pour la première fois dans des myes communes récoltées sur la Basse-Côte-Nord. En 2000, les glandes digestives des pétoncles des Îles-de-la-Madeleine demeuraient intoxiquées et des traces d'acide domoïque étaient mesurées dans des myes communes, moules bleues et couteaux de mer tout le long de la Côte-Nord du Saint-Laurent, de Tadoussac à Havre-Saint-Pierre. Conjointement aux données de l'inspection, le programme de monitorage des algues nuisibles de l'Institut Maurice-Lamontagne (Pêches et Océans Canada) a révélé la présence de deux espèces de diatomées potentiellement productrices d'acide domoïque dans le Saint-Laurent : *Pseudo-nitzschia seriata* et *Pseudo-nitzschia delicatissima*. L'examen des données a mis en évidence une coïncidence entre l'apparition de l'acide domoïque dans certains mollusques des Îles-de-la-Madeleine et de la Côte-Nord et la présence de *P. seriata* alors que des floraisons massives de *P. delicatissima* (en l'absence de *P. seriata*) n'occasionnent pas de toxicité. Les analyses en laboratoire réalisées sur une souche de *P. seriata* isolée de l'estuaire du Saint-Laurent lors d'un épisode de toxicité ont montré sa capacité à produire de l'acide domoïque alors que toutes les tentatives faites en ce sens avec *P. delicatissima* isolé d'autres régions de l'est du Canada se sont avérées négatives jusqu'à maintenant. Ces résultats récents indiquent que les floraisons de *P. seriata* dans le Saint-Laurent et les intoxications des mollusques par l'acide domoïque qui en résultent représentent un risque potentiel qui devra être considéré dans l'avenir.

1. INTRODUCTION

Historically, the estuary and Gulf of St. Lawrence are recognized as regions significantly affected by paralytic shellfish toxins due to recurrent blooms of the dinoflagellate *Alexandrium tamarense* (Prakash et al. 1973; Blasco et al. 1998). In August 1998, domoic acid was first detected in two samples of scallop gonads from the Îles-de-la-Madeleine. Over the next two years, domoic acid was also discovered in other areas, mainly on the north shore of the estuary and Gulf of St. Lawrence as well as the Îles-de-la-Madeleine, modifying the usual toxicity pattern of molluscs in maritime Quebec.

Domoic acid (Figure 1a) is a natural, water-soluble, thermostable amino acid (Wright et al. 1989) produced by certain red macroalgae (Takemoto and Daigo 1958; Impellizzeri et al. 1975) and by certain single-celled phytoplankton of the class diatom and genus *Pseudo-nitzschia* (Buck et al. 1992; Bates et al. 1998). This phycotoxin has an effect similar to the neurotoxic amino acid kainate (Figure 1b), both of which act as competitors of glutamate (Figure 1c), a neurotransmitter of the central nervous system. It operates by activating the kainate receptors of the hippocampus, thus generating neuro-excitatory activity that can lead to brain damage and, in large doses, even death (Debonnel et al. 1989; Quilliam and Wright 1989). Humans are poisoned by consuming molluscs that have in turn been contaminated by ingesting the toxic diatoms, which are part of their diet. In the first 24 hours, victims develop gastro-intestinal symptoms such as nausea, vomiting, anorexia, diarrhea, abdominal cramps and gastric bleeding. In the more severe cases, neurological disorders such as confusion, memory loss, disorientation and coma may occur, whence the acronym ASP, for "Amnesic Shellfish Poisoning," which designates this type of poisoning[1] (Perl et al. 1990; Todd 1993).

The first and most dramatic case of ASP occurred in Canada in the fall of 1987, when 107 people were poisoned after eating cultured blue mussels from Cardigan Bay, Prince Edward Island (Bates et al. 1989). The diatom *Nitzschia pungens* f. *multiseries*[2] was the species responsible for the poisoning (Bates et al. 1989). No other cases have since been reported in Canada. However, a total of nine species of *Pseudo-nitzschia* have now been recognized worldwide as domoic acid producers (Bates 2000), including *P. pseudodelicatissima* (Martin et al. 1990) and *P. seriata* (Lundholm et al. 1994). In Quebec, the current programs for monitoring molluscs and toxic algae make it possible to monitor the emergence of toxins and algae present in the estuary and one part of the Gulf of St. Lawrence. Two species of *Pseudo-nitzschia* present in these regions are liable to cause ASP: *P. delicatissima* and *P. seriata*. To date, however, it has not been possible to associate either of them with the presence of domoic acid. The goals of this study were, first, to note the extent and evolution of domoic acid contamination[3] in Quebec molluscs, and second, to establish a relation between blooms of *P. delicatissima* and *P. seriata* and the toxicity of molluscs collected at sampling sites common to both monitoring programs.

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1. The French acronym is IAM, for "Intoxication Amnestique par les Mollusques".
2. This species has been renamed *Pseudo-nitzschia multiseries* (Hasle 1995). It, as well as *P. pungens*, has never been observed at the MLI's eleven toxic algae monitoring stations.
3. In this report, the term "contamination" applies when values are obtained that are above the detection limit for the method used for domoic acid assays in molluscs.

2. MATERIALS AND METHODS

2.1. MOLLUSC SAMPLING

Since 1984, the Canadian Food Inspection Agency (CFIA) has been responsible for the program to monitor marine phycotoxins (algal toxins) in molluscs harvested for commercial or recreational purposes. Monitoring for domoic acid began sporadically in 1997 and became routine in 1998. The network of collection sites chosen for this purpose covers all of maritime Quebec. On the north shore of the St. Lawrence, it extends from Tadoussac to Natashquan on the Basse-Côte-Nord, and on the south shore, from Cacouna to Miguasha, around the Gaspésie. A number of sites in the Îles-de-la-Madeleine are also included in this network. There are nearly 75 sites in all, some 50 of which have been chosen for this study because they were sampled regularly between 1998 and 2000 (Figure 2a). Sampling is normally conducted on a weekly basis from April to November but can vary depending on site accessibility and resource availability. In addition to the CFIA's inspectors, various other stakeholders have a role in mollusc collection, including persons in the fishing and mariculture industries. The different species studied include soft-shell clams (*Mya arenaria*), razor clams (*Ensis directus*), scallops (mainly *Placopecten magellanicus* but also *Chlamys islandica*), blue mussels (*Mytilus edulis*) and whelks (*Buccinum undatum*), both wild and cultured. For each species, a minimum of 10 to 12 organisms are sampled so as to obtain the 150 g of tissue necessary for the toxicological analysis. After collection, the samples are refrigerated and shipped under conditions that allow for them to be kept alive until arrival at the laboratory.

2.2. MEASUREMENT OF MOLLUSC TOXICITY

The method used by the CFIA for the domoic acid (DA) assay is an adaptation of the method described by Gilgan et al. (1990). It is applied to molluscs, crustaceans or fish, whether alive, processed or frozen. The method begins with a methanol extraction on a homogenate of tissue to be analyzed. Five grams of homogenate are diluted with 5 mL of purified water. Ten mL of methanol are added to this mixture, which is then centrifuged. An aliquot of the supernatant is filtered, diluted and analyzed by reverse-phase high-performance liquid chromatography (HPLC) with UV detection. Toxin concentration is determined using certified standards from the National Research Council of Canada in Halifax, Nova Scotia. When the analyses were performed, the detection limit of this method was 0.2 μg DA g^{-1} tissue. In Canada, the regulatory limit has been set at 20 μg DA g^{-1} tissue (Gilgan et al. 1990). Consequently, when the toxicity of a mollusc is 20 μg DA g^{-1} tissue or higher, the shellfish growing area is immediately closed to harvesting. For the area to be reopened, it is necessary to obtain three consecutive results below the limit and showing a decreasing trend, over a 14-day period after obtaining the first result below 20 μg DA g^{-1} tissue. The CFIA decides on the target species, depending on the situation.

2.3. PHYTOPLANKTON SAMPLING

The Ocean Sciences Branch of the Maurice Lamontagne Institute (MLI) has been responsible for the Harmful Algae Monitoring Program for Quebec since 1989. The sampling network in place is comprised of eleven coastal stations covering all of maritime Quebec (Figure 2b). At each station, phytoplankton samples are taken every week from mid May to late October by contractors, collaborators or MLI employees. Two types of samples are collected. The first is a sample of seawater taken from the surface, using a pail. The water is filtered through a 250-μm mesh, and 500 mL is preserved by acidic Lugol's solution to a final concentration of 1% (v/v). The second sample is taken using a 2-m conical phytoplankton net installed on a metal ring 0.5 m in diameter. The net, made of 20 μm Nytex® (Tetko Inc. Briarcliff Manor, NY, USA) and ending with a 0.3 L codend, is hauled from the bottom to the surface so as to sample the entire water column. A subsample of 20 mL is preserved with acid Lugol's solution to a final concentration of 1% (v/v), and is representative of phytoplankton in the entire water column. In both cases, the acid Lugol's solution acts as a preservative of the phytoplankton cells.

2.4. PHYTOPLANKTON IDENTIFICATION AND ENUMERATION

Phytoplankton cells were identified and enumerated using a 500 mL sample of surface water. The phytoplankton samples were analyzed according to the Utermöhl method (1931), using an inverted optical microscope. Subsamples of 100 mL seawater treated with acid Lugol's solution were sedimented for 48 hours directly into 5-mL counting chambers. Cells were identified and enumerated over half of the surface of the chamber, in alternating transects. Using this method, one cell counted per 2.5 cm^2 corresponds to 20 cells L^{-1} (detection limit).

2.5. OVERLAP OF THE TWO MONITORING PROGRAMS

The mollusc and phytoplankton monitoring programs are implemented independently at separate stations. However, certain stations are considered common to both programs because of their proximity. This applies for Tadoussac, Baie-Comeau, Sept-Îles, Natashquan, Penouille, Gascons, Carleton and Havre-aux-Maisons (H-A-M) lagoon in the Îles-de-la-Madeleine (Table 1; Figure 2a, 2b). This overlap of sampling sites allows the information derived from each program to be interrelated. In this way, data collected between 1997 and 2000 have been compared by plotting the temporal evolution of domoic acid concentrations in molluscs and of *Pseudo-nitzschia* spp. blooms in seawater. However, only the sampling stations of Baie-Comeau, Sept-Îles, Natashquan and H-A-M lagoon have revealed interesting coincidences between toxicity values and measured cell abundances. The results from these four stations will be discussed in greater detail in the second part of the results section. For the other stations, the absence of toxicity, or the location of the station too far from where the toxic algae were sampled, did not allow us to establish any relations between toxicity and cell abundance.

2.6. *PSEUDO-NITZSCHIA SERIATA* CULTURES, SCANNING ELECTRON MICROSCOPY AND DOMOIC ACID ANALYSIS

One strain of *Pseudo-nitzschia seriata* was isolated from a sample taken on August 9, 2000 at the Mont-Joli monitoring station (MLI) and cultured there in f/2 medium (Guillard and Ryther 1992) at a temperature of 6°C under constant irradiance of 50-75 μmol photons m^{-2} s^{-1} produced by "Cool White" fluorescent lamps. In February 2001, the *P. seriata* culture was shipped to the Gulf Fisheries Centre in Moncton, New Brunswick, to confirm its identification by scanning electron microscopy and to verify the ability of this strain to produce domoic acid.

Prior to observation by scanning electron microscopy, the *P. seriata* samples were acid cleaned using the permanganate oxidation method of Hasle and Fryxell (1970). The cleaned cells were mounted on aluminum stubs, sputter coated with 20 nm of gold and observed with a JEOL JSM-5600 scanning electron microscope at the Digital Microscopy Facility (Mount Allison University, Sackville, NB).

The *P. seriata* culture was 10 days old when it was received on February 5, 2001. The cells were in the stationary growth phase and in poor physiological condition. Nevertheless, the domoic acid analysis was carried out on February 8. First, the cultured cells were broken by sonication and then filtered using a syringe-filter of 0.20 μm porosity (Advantec MFS Inc.) to remove cellular debris. The domoic acid concentration was then determined in the filtrate using a pre-column derivatization method with 9-fluorenylmethylchloroformate to obtain fluorenylmethoxycarbonyl (FMOC) derivatives, followed by reverse-phase HPLC with fluorescence detection (Pocklington et al. 1990). This highly sensitive method of analysis (detection limit of 0.5 ng mL^{-1}) is different from that described in Section 2.2 for molluscs and permits domoic acid analyses of *Pseudo-nitzschia* cells growing in cultures or found in seawater samples (Wright and Quilliam 1995).

3. RESULTS

3.1. SPATIO-TEMPORAL DISTRIBUTION OF DOMOIC ACID CONTAMINATION

The results relating to domoic acid concentration measured in Quebec molluscs between 1998 and 2000 generally showed an evolution in the extent of contamination. To simplify the data presentation, the results for one year are presented on one map, and only the maximum concentration detected at a collection site appears on the map (Figure 3a-c). In 1998, domoic acid was measured at low concentrations in two samples of sea scallop gonads from offshore of the Îles-de-la-Madeleine (Figure 3a). In 1999, high concentrations of domoic acid were measured in the digestive glands of scallops from the Îles-de-la-Madeleine: up to 24.6 $\mu g\, g^{-1}$ in the H-A-M lagoon and up to 585 $\mu g\, g^{-1}$ offshore (Levasseur et al. 2001)[4]. Lower concentrations, not exceeding 6.0 $\mu g\, g^{-1}$, were recorded mainly in soft-shell clams and in one sample of blue mussels

4. This report presents results of domoic acid concentrations found in digestive glands of Îles-de-la-Madeleine scallops for the year 1999. However, data not available at the time that this report was being prepared now indicate slightly higher maximum concentrations of domoic acid in the digestive glands of scallops from offshore and from the H-A-M lagoon.

from the Basse-Côte-Nord (Figure 3b). In 2000, high levels of domoic acid were again observed in the digestive glands of Îles-de-la-Madeleine scallops: up to 4.8 $\mu g\, g^{-1}$ in H-A-M lagoon and up to 265 $\mu g\, g^{-1}$ offshore. On the Côte-Nord, the contamination extended into estuaries, resulting in the detection of domoic acid, mostly at trace amounts (< 1.0 $\mu g\, g^{-1}$), at multiple locations (Figure 3c). Once again, domoic acid was detected mainly in soft-shell clams, but was also found in a few samples of blue mussels and razor clams. In 2000, trace amounts of domoic acid were recorded for the first time on the Gaspésie, in blue mussels grown in Maria, near Carleton (Figure 3c). Looking at the results generally, our attention is drawn to the contamination of scallops offshore of the Îles-de-la-Madeleine, not only because of the high domoic acid concentrations measured but also because of the persistence of the toxicity over many months (Figure 4). However, the absence of sampling for phytoplankton in scallop fishing areas offshore of the Îles-de-la-Madeleine prevents us from drawing a parallel between the presence of toxic algae and the toxicity measured in molluscs, as in the case of the stations presented in Section 3.2.

3.2. TEMPORAL EVOLUTION OF TOXICITY AND CELL ABUNDANCE AT FOUR COASTAL STATIONS

In Figures 5 to 8 in this section, the black dots on the x-axes indicate that an analysis was conducted but that the toxicity or cell abundance value was below the detection limit for the method of analysis used. The segments without dots represent periods when no sampling was conducted.

3.2.1. *BAIE-COMEAU STATION*

At the Baie-Comeau site, domoic acid was detected on two occasions in soft-shell clams in August 2000 (Figure 5). The two concentrations measured, although low at under 1.0 $\mu g\, g^{-1}$, were closely associated with dense blooms of *P. delicatissima* (1,191,400 cells L^{-1}) and *P. seriata* (70,080 cells L^{-1}). In August 1998, however, the abundance of *P. delicatissima* was nearly 700,000 cells L^{-1} without causing toxicity in the molluscs. This observation suggests that *P. delicatissima* is not toxic and hence that *P. seriata* could be responsible for the toxicity measured in August 2000. Nevertheless, smaller blooms of *P. seriata* (between 1,000 and 5,000 cells L^{-1}) recorded in 1998 and 1999 did not result in any mollusc toxicity (Figure 5).

3.2.2. *SEPT-ÎLES STATION*

At the Sept-Îles site, mollusc sampling was irregular over the four years. However, domoic acid was detected in some soft-shell clams in October 2000 (Figure 6). The low concentrations of domoic acid measured were preceded by relatively small blooms of *P. delicatissima* (103,353 cells L^{-1}) and *P. seriata* (2,240 cells L^{-1}). It seems highly unlikely that *P. delicatissima* was responsible for the toxicity since no domoic acid was detected in the weeks following, even though a bloom of nearly 1,800,000 cells L^{-1} occurred in August 1998. The observations made at Baie-Comeau suggest that the intensity of the *P. seriata* bloom documented at Sept-Îles was insufficient to generate the toxicity measured. It is possible that the weekly sampling was inadequate to record the real maximum of *P. seriata* cell numbers that might have explained the toxicity signal. In July and August 1997, *P. seriata* blooms of over 40,000 cells L^{-1} were recorded, but unfortunately, in this case as in that of July 1999, no mollusc analyses were carried out concurrently with these blooms (Figure 6).

3.2.3. *NATASHQUAN STATION*

At the Natashquan site, the somewhat sporadic sampling of molluscs nonetheless yielded a few interesting observations. The value of 0.89 μg DA g^{-1} measured in some soft-shell clams on July 10, 1999 is well synchronized with a *P. seriata* bloom of 14,000 cells L^{-1} on July 4 (Figure 7). The results show once again that dense blooms of *P. delicatissima* do not induce toxicity in soft-shell clams in the days that follow. Such was the case in July 1999 and October 2000, when concentrations of 135,000 and 212,000 cells L^{-1}, respectively, did not translate into a toxicity signal in the clams analyzed (Figure 7).

3.2.4. *HAVRE-AUX-MAISONS (H-A-M) LAGOON STATION*

At the H-A-M site, domoic acid was found primarily in the digestive glands of scallops and at higher concentrations than at the three previous sites (Figure 8). The findings here show two occasions of toxicity, in 1999 and 2000. The first occasion, which began on July 10, 1999, was preceded by a maximum bloom of 11,500 cells L^{-1} of *P. seriata* one month earlier, i.e. on June 8. At that time, the abundance of *P. delicatissima* was below 1,500 cells L^{-1}. The second occasion, which began on July 1, 2000, was preceded by a bloom of 1,500 cells L^{-1} of *P. seriata* two weeks earlier, whereas *P. delicatissima* did not appear in the water until after the domoic acid was detected. In 1999, it appears that the gap between the increase of *P. seriata* in the water and the first toxicity measurement was too large to relate the two. In 2000, the *P. seriata* bloom seems too small to explain the toxicity. However, a laboratory study comparing blue mussels (*Mytilus edulis*) and sea scallops (*Placopecten magellanicus*) (Wohlgeschaffen et al. 1992) has shown that the efficiency[5] of domoic acid accumulation is higher for sea scallops (5-100%) than for blue mussels (1-5%). This means that scallops retain a higher proportion of ingested domoic acid than do mussels. Furthermore, domoic acid is more persistent in the digestive glands of scallops, rendering the detoxification of this organism slower (Wohlgeschaffen et al. 1992; Douglas et al. 1997). It is therefore probable that, for a similar bloom of toxic cells, the toxicity signal is more substantial for scallops than for blue mussels or soft-shell clams, for example. For these reasons, it is conceivable that the toxicity measured on July 10, 1999, in scallops is related to the increased abundance of *P. seriata* one month earlier (Figure 8). In 2000, the more regular sampling of scallops produced a toxicity pattern that seems to reflect a slow detoxification occurring after a toxicity response following an increase of *P. seriata* in the seawater (Figure 9). The detoxification rate varies over time, from 0.166 $\mu g\, g^{-1} d^{-1}$ (~ 0.007 $\mu g\, g^{-1}\, h^{-1}$) on Day 1 to 0.016 $\mu g\, g^{-1}\, d^{-1}$ (~ 0.0007 $\mu g\, g^{-1}\, h^{-1}$) on Day 60. For an initial domoic acid concentration similar to ours, Whyte et al. (1995) measured laboratory rates in mussels (*Mytilus californianus*) of about 0.41 $\mu g\, g^{-1}\, h^{-1}$ at the start of detoxification and 0.01 $\mu g\, g^{-1}\, h^{-1}$ after 14 days. Our findings, although obtained in a natural environment where the uptake of domoic acid was not controlled, support the idea that scallops are much slower to detoxify than are blue mussels. The contamination results obtained at the H-A-M site seem to be a less intense reflection of the situation prevailing offshore of the Îles-de-la-Madeleine.

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5. Efficiency = Observed accumulation (in μg) / estimated accumulation (in μg) x 100

3.3 SCANNING ELECTRON MICROSCOPY AND DOMOIC ACID PRODUCTION

The scanning electron microscopy observations confirmed the presence of two species of *Pseudo-nitzschia* in the Gulf of St. Lawrence: *P. delicatissima* (Figure 10a, b) and *P. seriata* (Figure 10c-f). The identifications are based on the morphometric characteristics of the cells (Table 2) as compared with the data provided by Hasle and Syvertsen (1996).

One strain of *P. seriata*, isolated from the Mont-Joli monitoring station, produced a peak on the HPLC chromatogram at a retention time equivalent to that of domoic acid (Figure 11a). The domoic acid concentration was 10.8 ng mL^{-1}. Given a cell abundance in the culture sample of 13,800 cells mL^{-1}, the concentration of cellular domoic acid corresponds to 0.8 pg DA $cell^{-1}$. To confirm the presence of domoic acid in the culture, a culture sample was spiked with 19.0 ng DA mL^{-1}, using a domoic acid standard. The result was an increase in peak height, at the same retention time as previously obtained, and a total concentration of 25.2 ng DA mL^{-1} (Figure 11b).

4. DISCUSSION

The results show two very distinct situations of domoic acid contamination, namely contamination of Côte-Nord soft-shell clams and contamination of Îles-de-la-Madeleine scallops. In the former case, toxicity values were low and well within the permitted limit of 20 μg DA g^{-1}. Most of the time, these were trace amounts. In the latter case, the situation is more of a concern on account of the high concentrations of domoic acid measured in the digestive glands of the scallops. In July 1999, a public warning was issued by the Quebec Department of Agriculture, Fisheries and Food not to eat scallops whole, but to consume only the adductor muscle, which is generally not contaminated. The study by Douglas et al. (1997) on the sea scallop *Placopecten magellanicus* showed that domoic acid tissue concentrates in the following descending order: digestive gland >> remaining soft tissue >> adductor muscle. Even with a maximum concentration of 3,108 μg DA g^{-1}, which is 150 times the limit set by the regulations, only trace amounts of between 0.7 and 1.5 μg DA g^{-1} were observed at the same time in the adductor muscle during that study. In the natural environment, our results of measurements made on Îles-de-la-Madeleine scallops are consistent with the observations of Douglas et al. (1997). Even though the limit of 20 μg DA g^{-1} was often far exceeded in the digestive glands of offshore scallops, that threshold was never reached in the gonads, and the adductor muscles were never contaminated by domoic acid. For scallops farmed in the lagoon, the permitted limit was exceeded only once, in 1999, in the digestive glands, but the toxin was not detected in the gonads nor in the adductor muscles. This toxicity pattern, which mainly involved Côte-Nord soft-shell clams and Îles-de-la-Madeleine sea scallops, is the result of a greater emphasis in the sampling of soft-shell clams than of other molluscan species, and a different response to domoic acid by the scallops, as explained in the previous section. However, occasional detection in blue mussels and razor clams in the maritime area indicates that domoic acid contamination is not exclusive to Côte-Nord soft-shell clams or Îles-de-la-Madeleine scallops. In the Îles-de-la-Madeleine lagoons, scallops showed relatively high and persistent toxicity values compared with blue mussels and soft-shell clams, which are only occasionally contaminated by trace amounts. In this case, physiological factors that are still undetermined might be responsible for this interspecies difference observed in the lagoons. In the laboratory, less than 10% of the domoic acid ingested by blue mussels (*Mytilus edulis*) can be found in tissue (Wohlgeschaffen et al. 1992) whereas the comparable figure is 50.9% for the sea

scallop *Placopecten magellanicus* (Douglas et al. 1997). Furthermore, toxin retention in tissue is far more pronounced for the sea scallop than for the blue mussel, although the reasons for this difference are not known (Wohlgeschaffen et al. 1992).

The results obtained by plotting the temporal evolution of domoic acid at the Baie-Comeau, Sept-Îles, Natashquan and Havre-aux-Maisons stations strongly suggest that *Pseudo-nitzschia seriata* is the species responsible for the domoic acid found in St. Lawrence molluscs. Compared with *P. delicatissima*, *P. seriata* is generally not abundant in the toxic algae monitoring samples. However, a toxicity signal appears in molluscs when there is a substantial increase in the abundance of *P. seriata* in the water. On the other hand, substantial blooms of *P. delicatissima* have, on many occasions not translated into mollusc toxicity, suggesting that the cells in question were not toxic. To support our observations, it was necessary to establish the ability of the *P. seriata* and *P. delicatissima* strains in the St. Lawrence to produce domoic acid. A first examination by Bates et al. (1989) on a strain of *P. seriata* from Prince Edward Island showed that it was non-toxic. Subsequently, all attempts to culture other strains of *P. seriata* at the Gulf Fisheries Centre (DFO, Moncton) proved unsuccessful. In summer 2000, following the detection of domoic acid in soft-shell clams at Baie-Comeau, a strain of *P. seriata* was isolated from a sample taken at Mont-Joli during the same period and was successfully cultured. Laboratory analyses, which are related here, later confirmed the capacity of this strain of *P. seriata* to produce domoic acid (Figure 11), a first for a strain isolated from the St. Lawrence, and indeed a first in Canada. The ability of *P. seriata* to produce domoic acid had been established earlier by Lundholm et al. (1994) with certain strains from Denmark. Smith et al. (1990) had reported that a microalga called *Nitzschia actydrophila* (later renamed *Pseudo-nitzschia delicatissima*) was associated with the production of domoic acid. However *P. delicatissima* has never been associated with any toxic events (Skov et al. 1999). Strains from southern Nova Scotia and the Bay of Fundy have been repeatedly cultured and laboratory tested, but none have proven toxic, strengthening the idea that strains of *P. delicatissima* are innocuous. The next step will be to isolate and culture strains of *P. delicatissima* from the estuary or Gulf of St. Lawrence so as to confirm or refute the non-toxicity of this species. The results obtained are preliminary and based on a limited amount of data, which do not allow one to predict the critical threshold of *P. seriata* cell abundance necessary to generate a toxicity signal in molluscs. Nevertheless, those results do shed light for the first time on the causal connection between *P. seriata* and the appearance of a domoic acid toxicity signal in St. Lawrence molluscs.

CONCLUSIONS

1. Domoic acid assays revealed that many species of molluscs may be affected by this phycotoxin, which can be found throughout maritime Quebec.

2. The concentrations measured in soft-shell clams, blue mussels and razor clams remained well below the regulatory limit of 20 μg DA g^{-1}. This limit was far exceeded in the digestive glands of scallops offshore of the Îles-de-la-Madeleine, although without any consequences for the scallop adductor muscle, which is the part that is most often consumed.

3. The results showed a coincidence between an increase in abundance of the diatom *Pseudo-nitzschia seriata* in seawater and the appearance of a domoic acid toxicity signal, whereas dense blooms of *P. delicatissima* did not induce mollusc toxicity.

4. Preliminary laboratory analyses by FMOC-HPLC have shown the capacity of a strain of *Pseudo-nitzschia seriata* isolated from the St. Lawrence to produce domoic acid whereas this demonstration has not be made for strains of *P. delicatissima*.

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Table 1. Overlap of mollusc collection sites and of toxic algae monitoring stations, by region, in Quebec.

Region of Quebec	CFIA mollusc collection site	MLI toxic algae sampling station
Côte-Nord	Pointe Rouge	Tadoussac
Côte-Nord	Rivière-Mistassini	Baie-Comeau
Côte-Nord	Baie-des-Forges	Sept-Îles
Côte-Nord	Île Michon	Natashquan
Gaspésie	Rivière-Saint-Jean Estuary	Penouille
Gaspésie	Port-Daniel Bay	Gascons
Îles-de-la-Madeleine	Central H-A-M lagoon	H-A-M lagoon

Table 2. Morphometric data for *Pseudo-nitzschia* species isolated from the estuary and Gulf of St. Lawrence. Values in parentheses are from Hasle and Syvertsen (1996).

Characteristic	*P. delicatissima*	*P. seriata*
Length (μm)	41 - 56	91 - 147
	(40 - 76)	(91 - 160)
Width (μm)	1.6 - 2.2	4.1 - 6.6
	(2.0 - 2.0)	(5.5 - 8.0)
Striae (in 10 μm)	38 - 40	15 - 19
	(36 - 40)	(14 - 18)
Fibulae (in 10 μm)	22 - 29	15 - 18
	(19 - 25)	(14 - 18)
Poroids (rows)	2	3 - 4
	(2)	(3 - 5)
Central interspace	present	absent
	(present)	(absent)

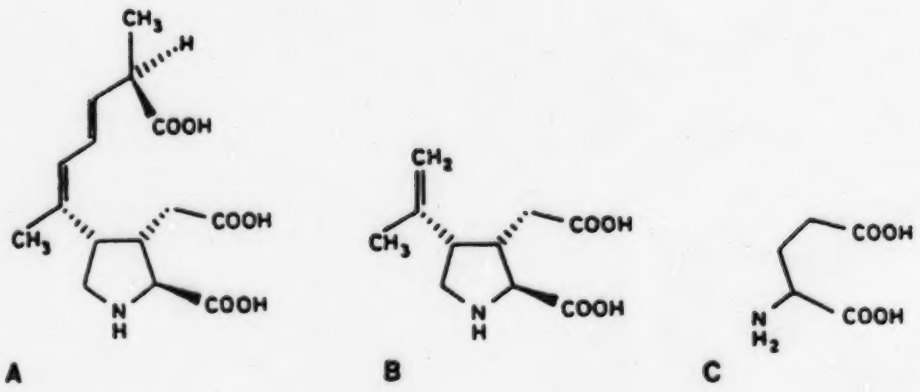

Figure 1. Stereochemical representation of: (a) domoic acid, (b) kainate and c) glutamate (from Skov et al. 1999, with the authors' permission).

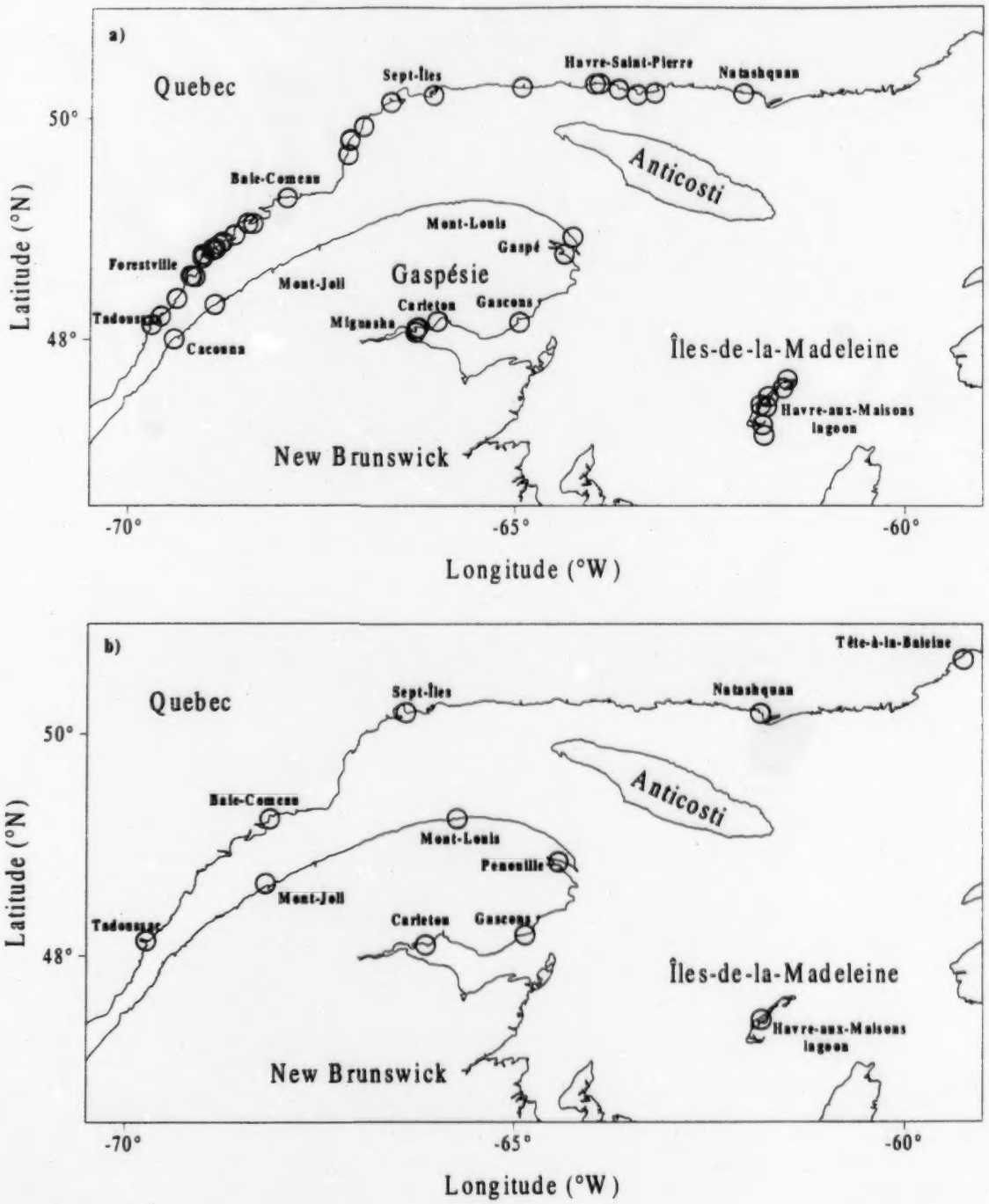

Figure 2. Locations of: (a) CFIA mollusc collection sites for domoic acid (only sites sampled regularly between 1998 and 2000 are shown) and (b) MLI toxic algae monitoring stations.

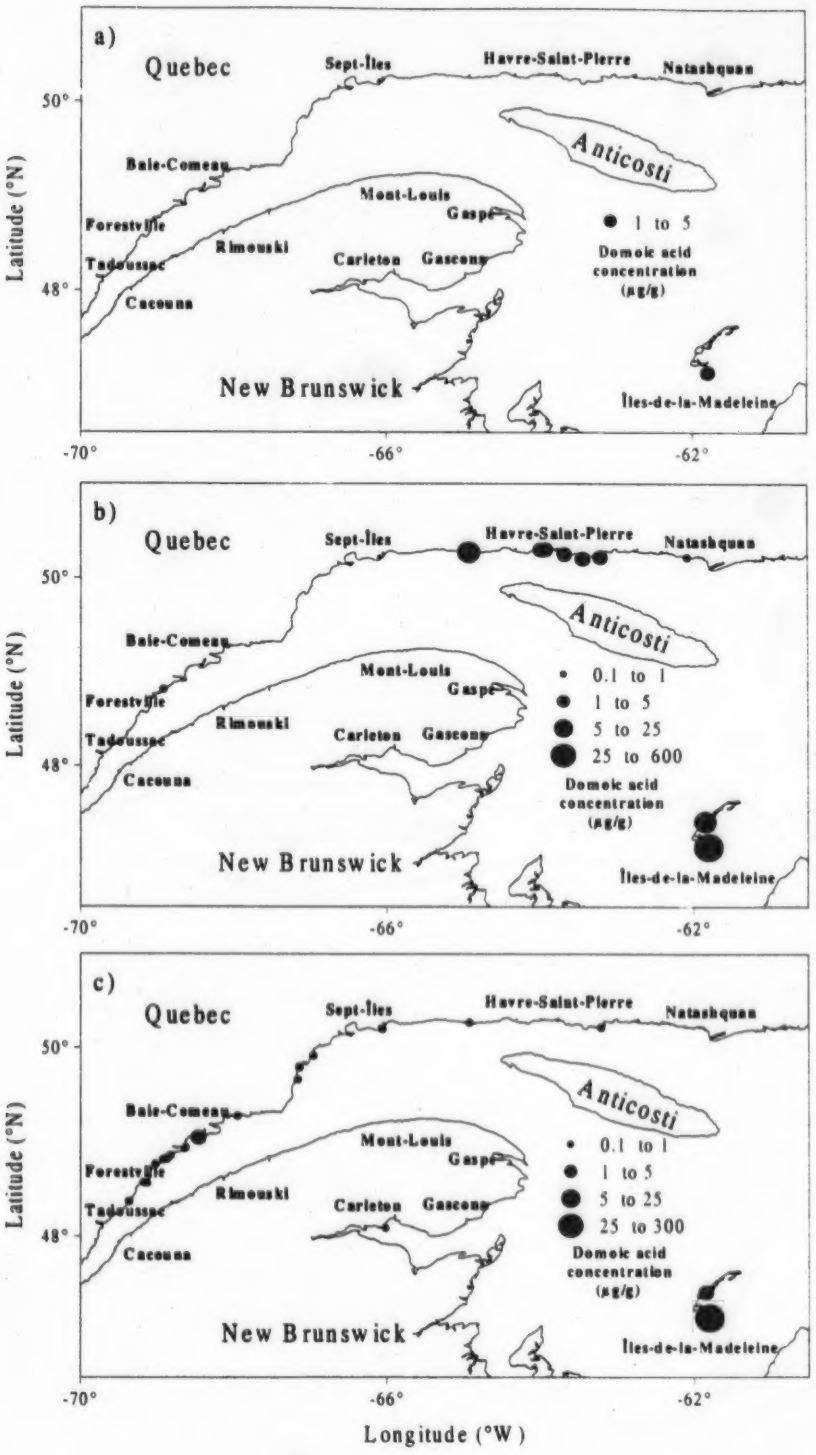

Figure 3. Evolution of domoic acid contamination at CFIA mollusc collection sites in the years (a) 1998, (b) 1999 and (c) 2000 (only maximum concentrations are shown).

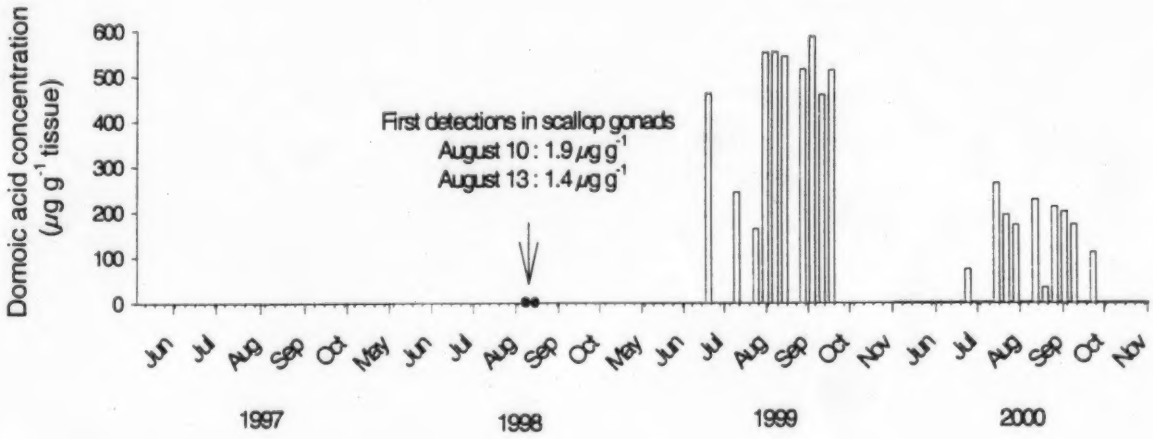

Figure 4. Temporal evolution of domoic acid concentration in digestive glands of scallops from offshore of the Îles-de-la-Madeleine between June 1997 and November 2000.

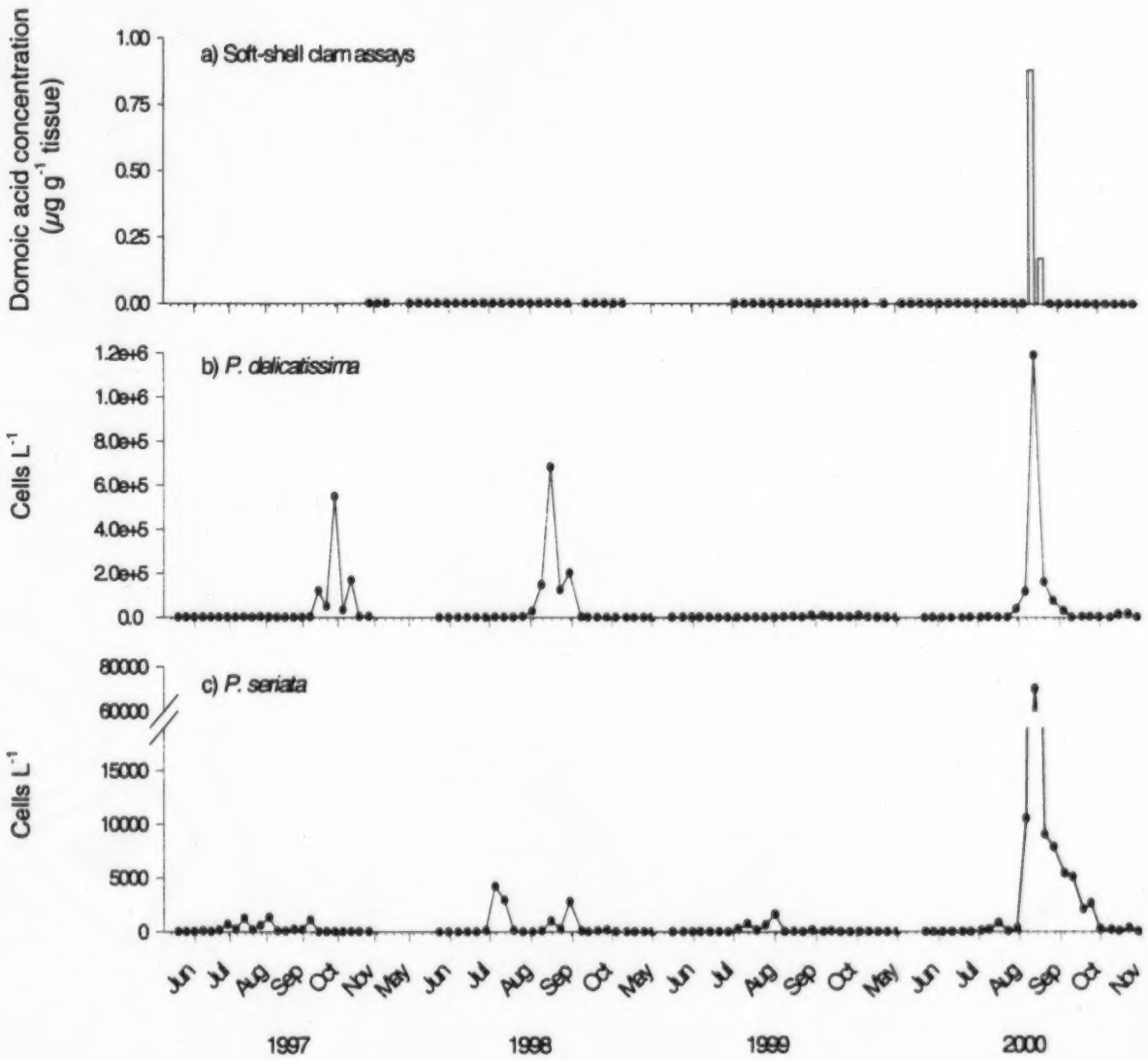

Figure 5. Temporal evolution of: (a) domoic acid concentration measured in soft-shell clams, (b) abundance of *Pseudo-nitzschia delicatissima* and (c) abundance of *Pseudo-nitzschia seriata* at the Baie-Comeau site between June 1997 and November 2000.

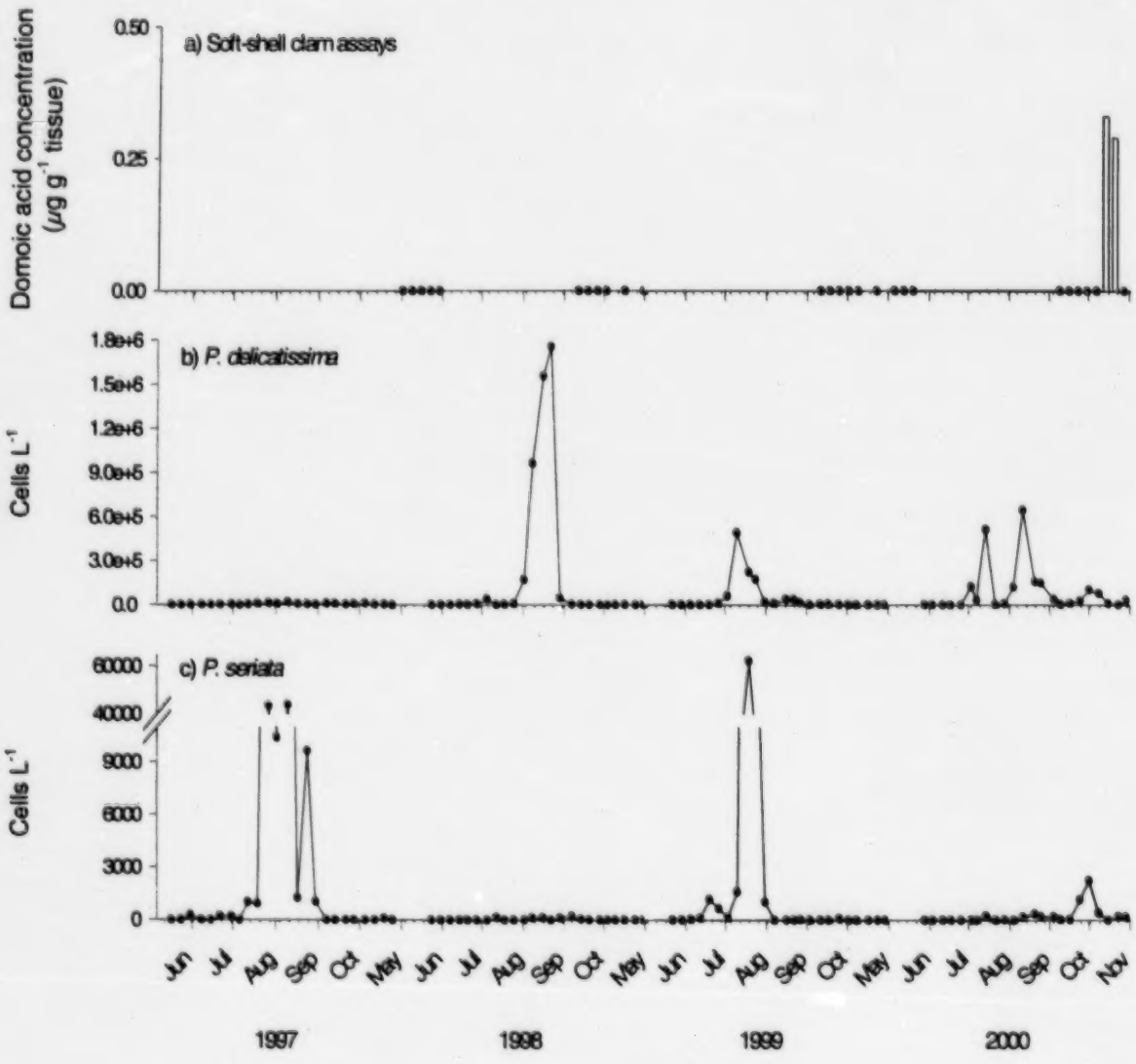

Figure 6. Temporal evolution of: (a) domoic acid concentration measured in soft-shell clams, (b) abundance of *Pseudo-nitzschia delicatissima* and (c) abundance of *Pseudo-nitzschia seriata* at the Sept-Îles site between June 1997 and November 2000.

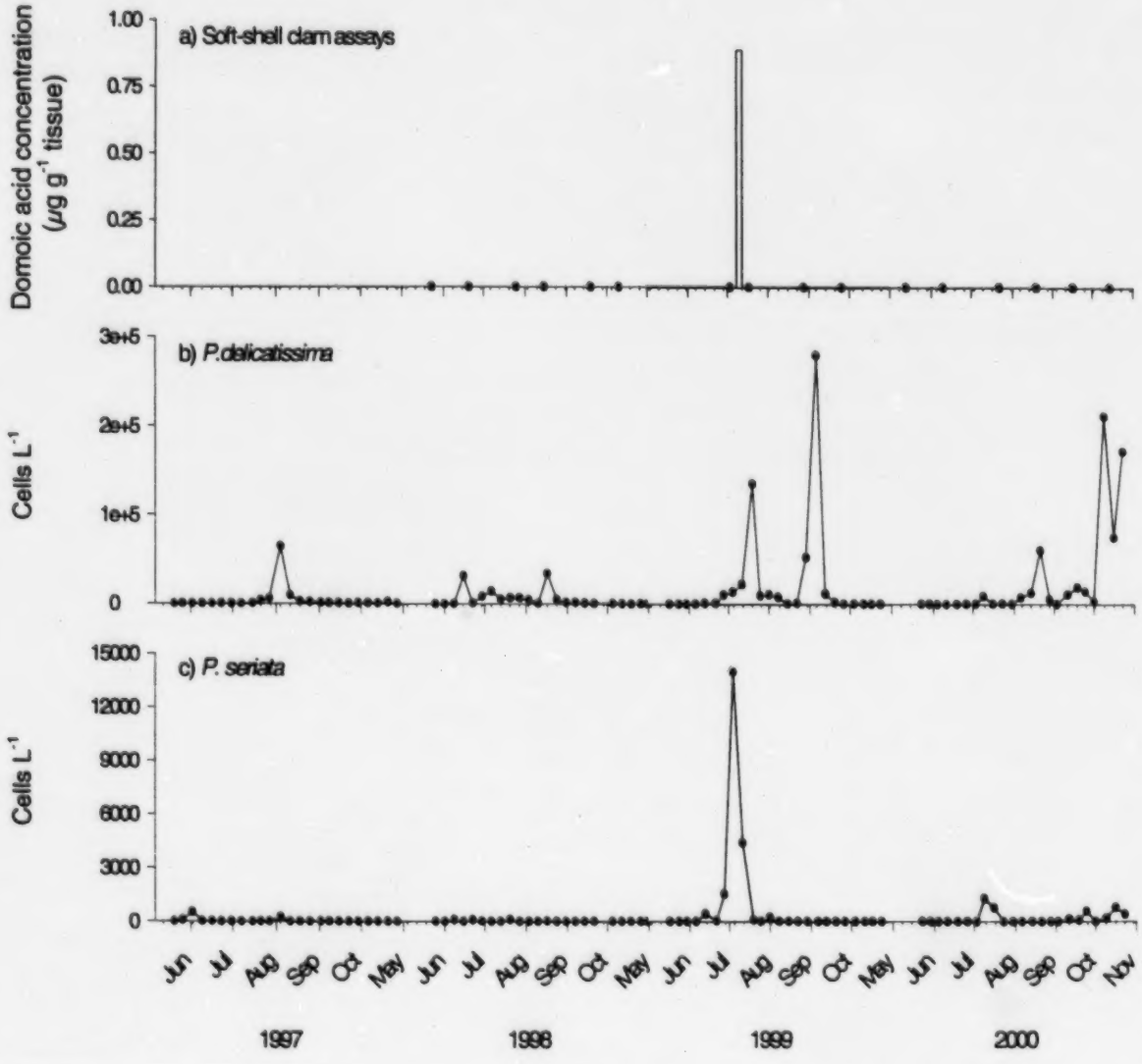

Figure 7. Temporal evolution of: (a) domoic acid concentration measured in soft-shell clams, (b) abundance of *Pseudo-nitzschia delicatissima* and (c) abundance of *Pseudo-nitzschia seriata* at the Natashquan site between June 1997 and November 2000.

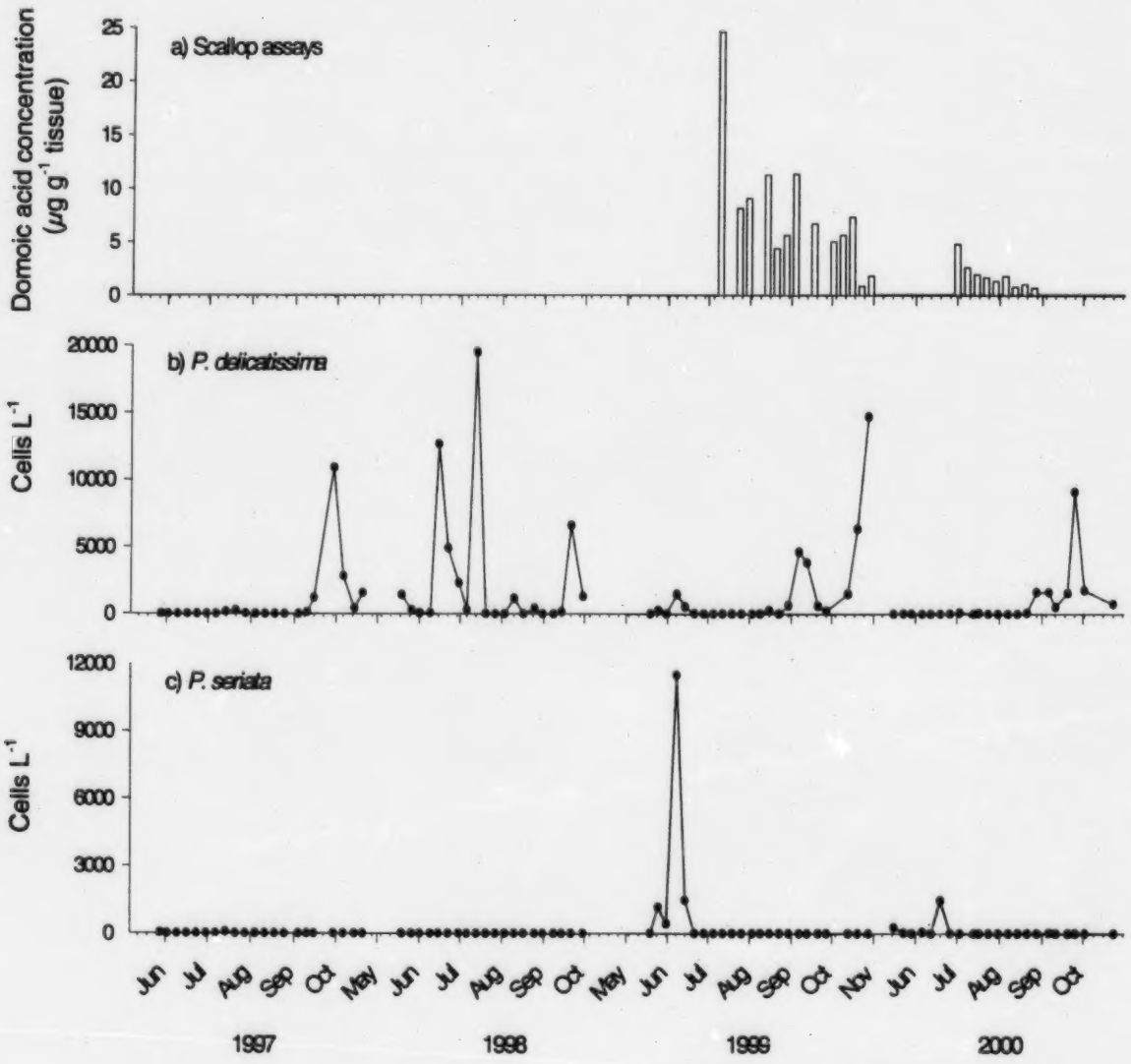
Domoic acid concentration (µg g-1 tissue)
a) Scallop assays
b) P. delicatissima
Cells L-1
c) P. seriata
Cells L-1
Jun Jul Aug Sep Oct May Jun Jul Aug Sep Oct May Jun Jul Aug Sep Oct Nov Jun Jul Aug Sep Oct
1997
1998
1999
2000

Figure 8. Temporal evolution of: (a) domoic acid concentrations measured in digestive glands of scallops, (b) abundance of *Pseudo-nitzschia delicatissima* and (c) abundance of *Pseudo-nitzschia seriata* at the Havre-aux-Maisons lagoon site in the Îles-de-la-Madeleine between June 1997 and November 2000.

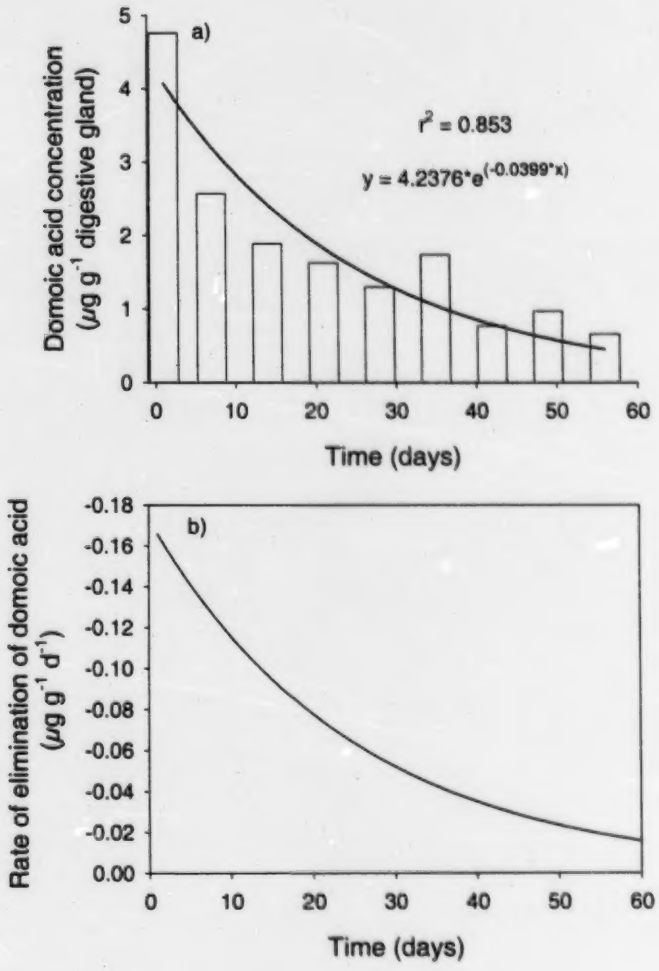

Figure 9. (a) Elimination of domoic acid from digestive glands of scallops from the Havre-aux-Maisons lagoon in the Îles-de-la-Madeleine during summer 2000 (curve indicates relation between domoic acid concentration and time) and (b) change in the rate of elimination of domoic acid from digestive glands of scallops, based on data presented in (a).

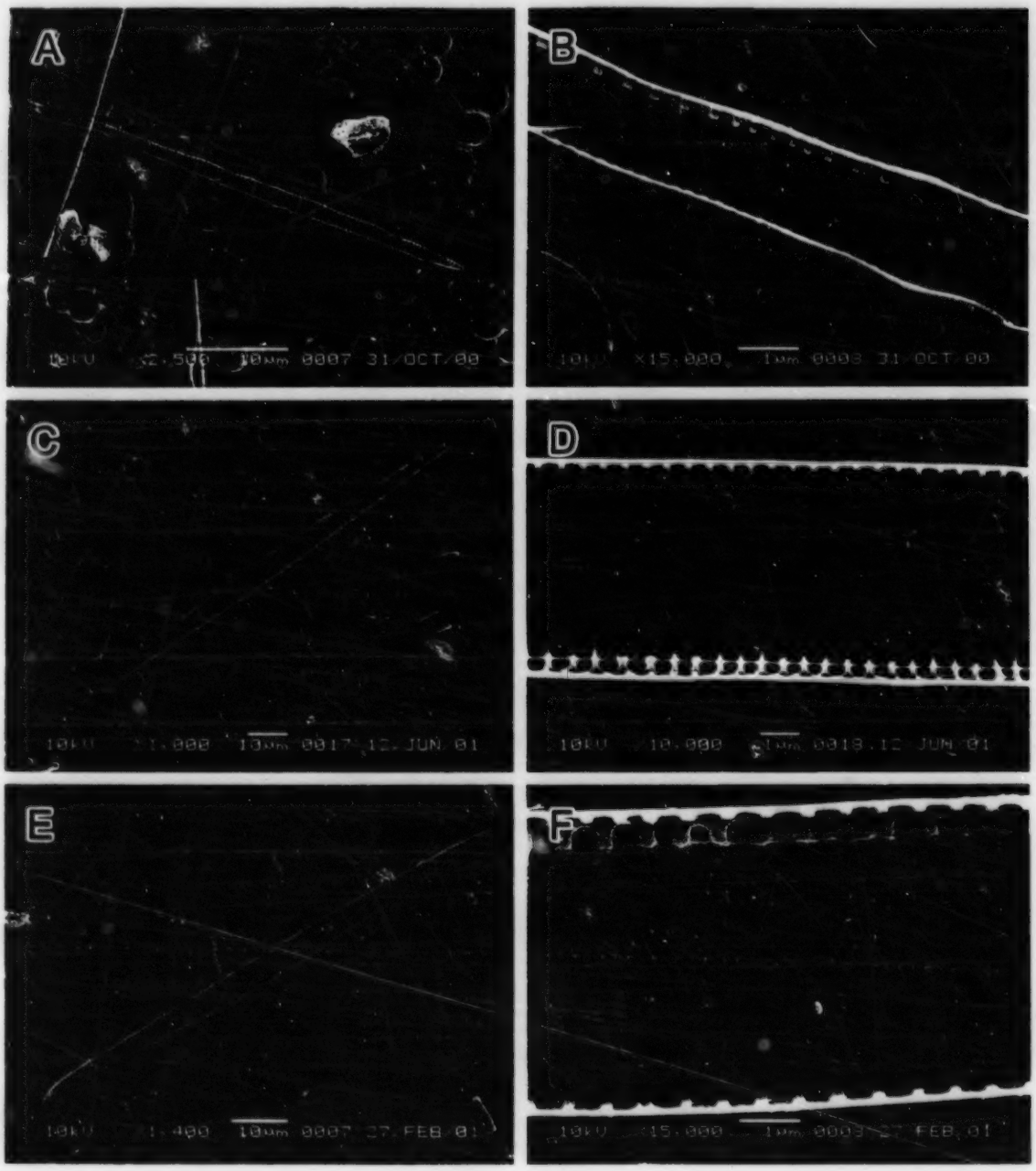

Figure 10. Scanning electron micrographs of *Pseudo-nitzschia* species isolated from the estuary and Gulf of St. Lawrence: (a, b) *P. delicatissima* from the Mont-Joli station (MLI); (c, d) *P. seriata* from the Havre-aux-Maisons station; (e, f) *P. seriata* from the Mont-Joli station (MLI).

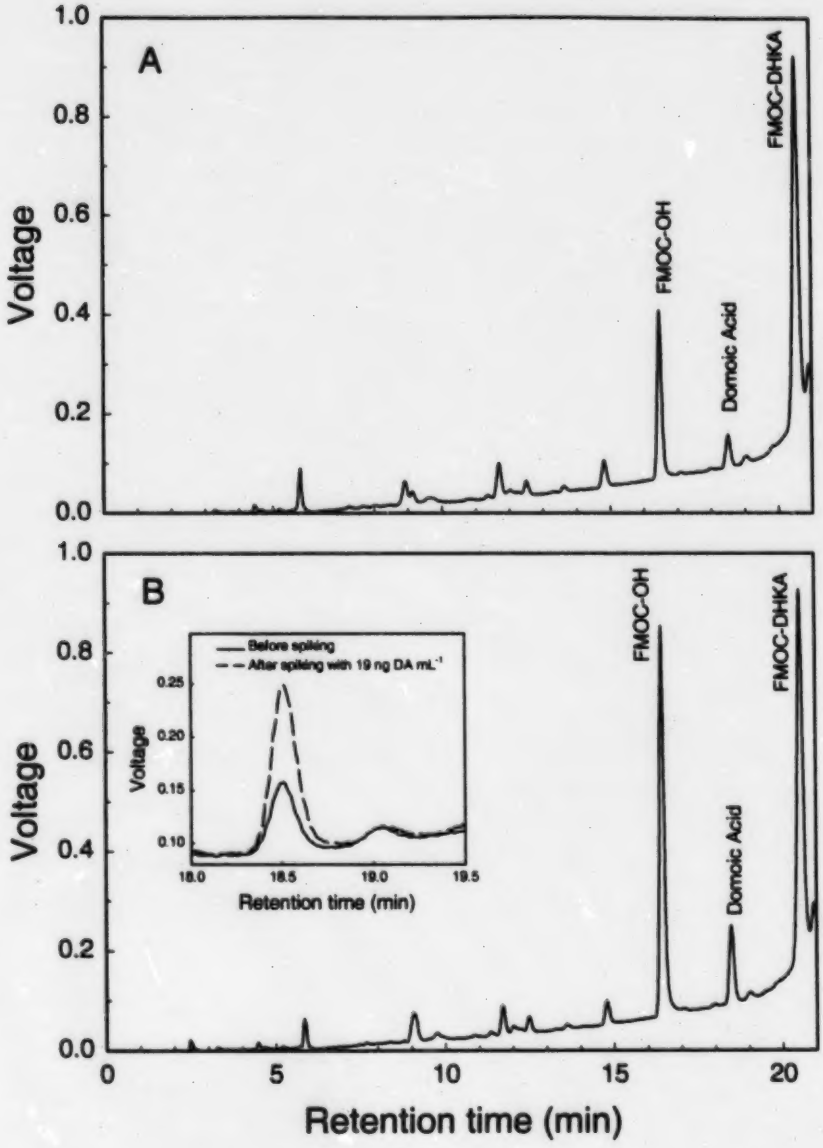

Figure 11. High-performance liquid chromatography of a culture of *Pseudo-nitzschia seriata* isolated from the Mont-Joli station (MLI). The FMOC-DHKA peak is the internal standard. The FMOC-OH peak is the result of an interaction between the excess FMOC reagent and water, and can vary in height for each sample. (a) Sample before spiking with a domoic acid standard and (b) sample after addition of 19 ng DA mL^{-1}, resulting in an increase in the domoic acid peak height at the same retention time. Inset: details of the chromatogram showing the identical retention time of the original peak and the peak after spiking with domoic acid.

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